Comparisons of Two By-Products and a Prepared Diet as Organic Fertilizers on Growth and Survival of Larval Paddlefish, *Polyodon spathula*, in Earthen Ponds

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ABSTRACT. Two agro-industrial by-products, rice bran (RB) and distillers dried solubles (DS), and a prepared diet (PD) were evaluated as organic fertilizers for the production of juvenile paddlefish in nine 0.02-ha earthen ponds over a 40-day culture period. Paddlefish yield from ponds fertilized with RB (209 kg/ha) was significantly greater ($P \le 0.05$) than that from ponds fertilized with DS (129 kg/ha), but it was not significantly greater than yields from ponds fertilized with PD (258 kg/ha). Fish survival from ponds fertilized with PD (79%) was significantly higher than from ponds fertilized with RB (55%) or DS (50%). There was no significant difference in

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survival between ponds fertilized with RB and DS. Secchi disk visibilities in ponds fertilized with RB were significantly lower than in ponds fertilized with DS and PD. Relatively low Secchi disk visibilities in RB-fertilized ponds were because of a brown stain or coloration which reduced sunlight penetration and growth of filamentous algae, not observed in DS- or PD-fertilized ponds. Larvae congregated in areas where PD was being applied, which suggested direct feeding on PD. Paddlefish did not respond when RB and DS were applied to ponds. Cost per juvenile paddlefish raised in ponds fertilized with RB was \$0.004, cheaper than \$0.011 for fish raised in ponds fertilized with PD or DS. Rice bran is the recommended agro-industrial by-product to raise juvenile paddlefish greater than 120 mm total length based on improved fish yields, pond water quality, and lower cost per fish. The prepared diet may be used not only as an organic fertilizer, but also as a supplemental feed.

INTRODUCTION

Paddlefish, Polyodon spathula, is valued as a commercial fish for both its flesh and its roe and also as a sport fish (Carlson and Bonislawsky 1981). However, paddlefish are listed as a special concern species in 23 states of United States (Williams et al. 1989). Continued loss of its riverine habitat and over-exploitation by commercial fisheries have been the principle threats to endemic paddlefish populations (Williams et al. 1989). Many state and federal fishery agencies are developing mitigation and restoration programs which require the stocking of cultured paddlefish to increase the population in its native range. Paddlefish culture is also being investigated because the fish are considered a desirable food fish (Semmens and Shelton 1986). The flesh is firm and completely boneless, making it popular to consumers (Decker et al. 1991). Proven techniques of raising larvae to juveniles (>120 mm total length) are an integral part of establishing a consistent supply of paddlefish to meet repopulation and culture demands.

Young fish less than 120 mm total length (TL) are particulate feeders and consume large cladocerans, especially *Daphnia* (Michaletz et al. 1982). Large cladocerans must be available during the first two weeks post-stocking for reliable paddlefish production (Mims et al. 1991). Thereafter, older paddlefish greater than 50 mm TL can feed on less preferred food items-small cladocerans and chironomids (Michaletz et al. 1983; Mims et al. 1991).

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Various types, quantities, and combinations of organic fertilizers have been used to stimulate zooplankton (primarily cladocerans) production in paddlefish culture ponds and to maintain suitable water quality for growth and development of the fish (Graham et al. 1986). Michaletz et al. (1982) reported that paddlefish larvae stocked at 49,000/ha reached 120 mm TL, with a survival of 16% after 80 days in ponds that were fertilized with a combination of brewer's yeast (453 kg/ha), alfalfa meal (227 kg/ha), dehydrated cow manure (453 kg/ha), and 10 bales of clover hay. Semmens (1982) stocked 49,000 larvae/ha into ponds that were fertilized with alfalfa pellets (600 kg/ha) and meat/bone meal (300 kg/ha) and inoculated with *Daphnia* spp.; after 40 days, he harvested fish that were about 100 mm, and survival averaged 58%. Recently, Mims et al. (1991) reported that ponds stocked at 61,775 larvae/ha, fertilized with rice bran (1,742 kg/ha) and liquid inorganic fertilizer (10-34-0; 69.5 L/ha), and inoculated with Daphnia pulex (0.5/L) produced juvenile paddlefish greater than 120 mm TL, with an average survival rate of 77% after 40 days. A high stocking density combined with organic and inorganic fertilization and an inoculation with preferred food organisms increased the number of paddlefish harvested by more than 35% and the yield by more than 128%. compared to results by Michaletz et al. (1982) and Semmens (1982). Further improvements in larval survival and juvenile yields from earthen ponds may be possible by finding organic fertilizers that, when combined with inorganic fertilizer and applied at the proper rates, can increase the production of Daphnia spp. and provide more food for the paddlefish.

The objectives of this study were to compare two commercially available agro-industrial by-products and a prepared diet as organic fertilizers to improve paddlefish survival and yield in earthen ponds and to determine the impact of these organic fertilizers on selected water quality variables over a 40-day period.

MATERIALS AND METHODS

Nine 0.02-ha earthen ponds located at Kentucky State University Aquaculture Research Center, Frankfort, Kentucky were used. Three ponds were randomly assigned to each of three treatments. Ponds received a pre-flooding treatment with liquid Hydrothol^{®1} at a rate of 0.2 mg/L to control filamentous algae. On April 12, 1989, ponds were filled to an average depth of 1.1 m with water taken from a surface water reservoir; the water was filtered through $385-\mu$ saran cloth socks.

Two commercially available agro-industrial by-products-rice bran (RB) and distillers dried solubles (DS)-and a prepared diet (PD)-Purina trout chow starter (#5100)-were evaluated as organic fertilizers. Fertilizer quantities and application schedules for each treatment were based on the nitrogen content of RB (control) as described by Mims et al. (1991) (Table 1). Total amount of nitrogen applied to each pond via organic fertilizers was 43 kg/ha (3,911 μ g/L). Organic fertilizers were analyzed as described by Horwitz (1980); carbon to nitrogen (C:N) ratios were determined with an elemental analyzer for macrosample (LECO CHN, model 600, St.Joseph, Michigan) (Table 2). Each pond received an additional 11 kg/ha (933 μ g/L) of nitrogen in the form of liquid ammonium polyphosphate (10-34-0) applied at varied amounts over the experimental period (Table 1). The weekly quantities of nitrogen and phosphorus $(\mu g/L)$ added to the ponds in the form of organic and inorganic fertilizers are reported in Table 3.

Ponds were inoculated with zooplankton, predominately *Daphnia* spp., at a rate of about 125,000 crustaceans per pond on April 13, 14, and 15. Each pond was equipped with one continually operated 5-cm polyvinyl chloride air-lift pump to provide thorough mixing of nutrients and zooplankton during the study (Parker 1979).

Paddlefish larvae, produced from broodfish collected in Kentucky, were held in 50-cm square boxes with window screen ($800-\mu$ mesh size) bottoms for 8 days after hatching until mouth parts were well developed, peristalsis had begun, and larvae were actively seeking food (Graham et al. 1986). Larvae averaging 16.8 mm TL (SD = 0.3 mm) and 23.0 mg (SD = 2.1 mg) were stocked on April 27 at 61,775 larvae/ha. Ten paddlefish were collected by seining from each pond weekly. The fish were measured in centimeters for TL and in grams for weight. Fish were harvested after 40 days. An

^{1.} Use of trade or manufacturer names does not imply endorsement.

TABLE 1. Organic and inorganic fertilization rates for 0.02-ha ponds that were stocked with 61,775 larval paddlefish/ha. Organic fertilizers were divided into the indicated number of applications. All treatments received the same amount of inorganic fertilizer (10-34-0). Three weekly applications of fertilizers were applied to the filled ponds during a two-week period prior to stocking (week 0).

Week	No. of applications	Rice bran (kg/ha/week)	Distillers dried solubles (kg/ha/week)	Prepared diet (kg/ha/week)	inorganic fertilizer (l/ha/week)	
0	6	1,410	600	345	37.0	
1	3	310	132	76	4.6	
2.5	8	157	67	39	9.3	
Total	17	2,348	1,000	577	78.8	

TABLE 2. Analysis of organic fertilizers applied to 0.02-ha ponds that were stocked with 61,775 larval paddlefish/ha.

Composition	Rice bran	Distillers dried solubles	Prepared diet
Crude Protein (%)	11.4	27.1	47.0
Fat (%)	14.1	6.2	24.7
Crude Fiber (%)	12.8	3.5	1.1
Moisture (%)	9.5	4.4	7.5
Phosphorus (%)	1.5	1.3	1.6
Potassium (%)	1.5	1.6	0.6
Magnesium (%)	0.8	0.5	0.2
Calcium (%)	1.1	0.1	2.5
C:N ratio	22:1	10:1	6:1

TABLE 3. Weekly nitrogen (N) and phosphorus (P) for organic and inorganic fertilizers, in mg/l applied to 0.02-ha ponds that were stocked with 61,775 larval paddlefish/ha. Ratios were calculated from total N and P from the combination of the organic and inorganic fertilizers.

				Organi	c fertilizers				
	No. of	Rice	Lbran		illers olubles		pared iet		ganic lizer
Week	applications	N	P	N	P	N	P	N	P
0	6	2,336	295	2,336	600	2,358	407	467	691
1	3	519	65	519	132	519	69	58	85
2-5	8	1,056	132	1,056	268	1,056	184	488	173
Total	17	3,911	492	3,911 1	1,000	3,911	680	993	1,468
Retio		2.5	: 1	2.0 :	: 1 -	2.3	9:1		

additional 40 fish were sampled at harvest for final individual TL and weight.

Water Quality Analysis and Management

Dissolved oxygen and water temperature, (polarographic dissolved oxygen meter and thermistor, YSI model 54A) and pH (Omega pHH-43 meter) were measured at 0700 and 1500 daily in each pond. Secchi disk visibility was measured daily at 0700. Water samples were collected weekly from each pond before fertilizers were applied and analyzed for alkalinity (as mg/L of CaCO₃), ammonia-nitrogen (NH₃-N), nitrite-nitrogen (NO₂-N), nitrate-nitrogen (NO₃-N), and total filterable orthophosphate (PO₄-P) (Hach DREL/5). Chlorophyll *a* was extracted from water samples with acetone and measured spectrophotometrically (APHA et al. 1980). Emergency aeration was provided by a 0.33-hp surface aerator whenever the dissolved oxygen concentration was predicted by graph (Boyd 1979) to decline below 40% of saturation (Andrews et al. 1973). Ponds fertilized with DS or PD were treated with 0.1 mg/L Hydrothol[®] on week 5 to control filamentous algae. Eleven kg of sodium chloride were added to each pond to prevent possible ni-trite-induced anemia (Tucker et al. 1989).

Statistical Analyses

Differences in survival and yields were assessed by analysis of variance (ANOVA) for a completely randomized design (SAS Institute, Inc. 1990). Paddlefish size and water quality data observed weekly in the ponds were assessed by a repeated measures version of ANOVA (split plot design). Mean differences between selected treatment means (*a priori* comparisons) were tested with contrasts. Weekly trends in water quality variables were tested using orthogonal polynomial contrasts. The probability level for tests was 0.05.

RESULTS

Fish Yield and Survival

During week 5, growth of filamentous algae in one DS pond could not be controlled with Hydrothol.[®] As a result, fish became entangled, and severe mortality occurred although it could not be accurately recorded. Because of low fish yield and survival, data in this pond were excluded from the analysis.

At harvest, mean paddlefish yield in ponds fertilized with RB was significantly greater than in ponds fertilized with DS, although the difference in survival was not significant (Table 4). Mean yield in ponds fertilized with RB were not significantly different than that from ponds fertilized with PD. However, mean survival in ponds fertilized with PD was significantly higher than that from ponds fertilized with RB.

Growth of the fish from the different treatments is shown in Figure 1. Statistical differences in fish TL and weight from ponds fertilized with RB or DS were noted on weeks 5 and 6 after stocking. Fish sampled from ponds fertilized with RB were significantly longer and heavier than fish from ponds fertilized with DS. Fish sampled from ponds fertilized with PD or RB were not significantly TABLE 4. Mean yield, growth rate, and survival of paddlefish larvae (\pm SE) stocked at 61,775/ha and cultured for 40 days in 0.02-ha ponds fertilized with rice bran, distillers dried solubles, or a prepared diet. Values followed by the same letter in each column are not significantly different at P > 0.05.

Treatment	Yield (kg/ha)	Growth rate (mm/d)	Survival (%)	
Rice bran	219±22a	2.8±0.2a	55±5b	
Distillers dried solubles	129±27b	2.3±0.1b	50±6b	
Prepared diet	258+228	2.6±0.1a	79±58	

different in size at all sampling dates, despite higher survival in ponds fertilized with PD. Total length and weight in each treatment increased linearly over weeks. At harvest, fish sampled in ponds fertilized with RB and PD averaged 130 and 120 mm TL, respectively, whereas DS fish averaged only 105 mm TL.

Water Quality

Mean Secchi disk visibilities for the different treatments are shown in Figure 2. Secchi disk visibilities for the test period in ponds fertilized with RB (61 cm) were significantly lower than those in ponds fertilized with DS (91 cm) or with PD (86 cm). Weekly Secchi disk visibilities in RB ponds were significantly lower than those in PD ponds on weeks 2, 3, and 4 or in DS ponds on weeks 2, 4, and 5. Mean phytoplankton abundance, as measured by chlorophyll-*a* concentrations, in ponds fertilized with DS (40 μ g/L) was significantly higher than that in RB (16 μ g/L) and in PD (20 mg/L) ponds. Weekly chlorophyll-*a* concentrations are illustrated in Figure 3. The low water transparency and low chlorophyll-*a* in RB ponds were due to a brown coloration or stain imparted by the RB. There were no statistical differences in phosphorous levels among treatments. Because of the water clarity in ponds fertilized with DS or PD, filamentous algae became established.

Mean alkalinity for ponds fertilized with RB (97 mg/L) was significantly higher than those fertilized with DS (87 mg/L) or with PD (84 mg/L). Mean pH for ponds fertilized with PD (8.4) was significantly higher than that in ponds fertilized with RB (7.8) or

FIGURE 1. Mean weekly total lengths (A) and weights (B) of paddlefish in ponds fertilized with rice bran, distillers dried solubles, or prepared diet and the estimated linear increases. Mean total length and weight at stocking were 17 mm and 23 mg, respectively.

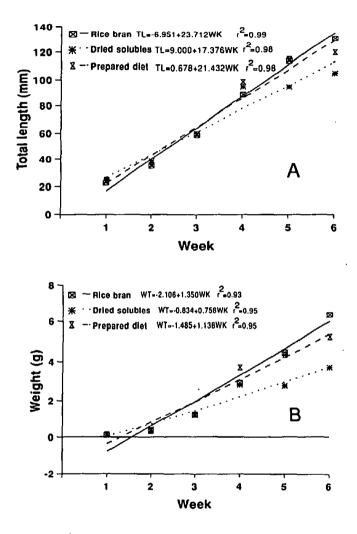
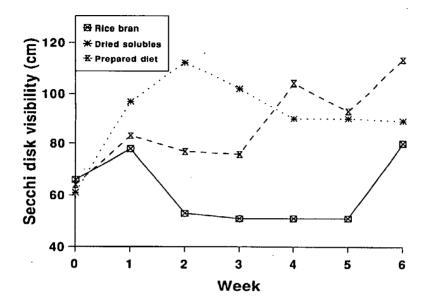


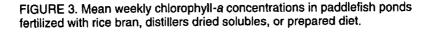
FIGURE 2. Mean weekly Secchi disk visibilities in paddlefish ponds fertilized with rice bran, distillers dried solubles, or prepared diet.

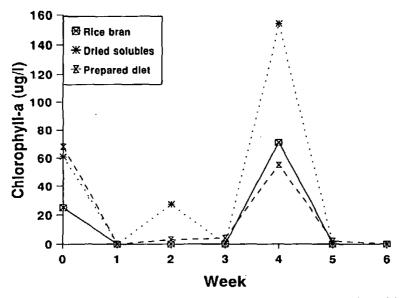


with DS (8.0). Mean weekly pH for the different treatments are shown in Figure 4. Weekly pH in ponds fertilized with RB was significantly lower on weeks 1, 3, and 4 than that in PD ponds, and it was significantly lower on week 4 than that in DS ponds.

Mean early morning dissolved oxygen in ponds fertilized with RB (7.1 mg/L) was significantly lower than that in ponds fertilized with DS (7.7 mg/L); the average dissolved oxygen of RB and DS ponds (7.4 mg/L) was significantly lower than in ponds fertilized with PD (8.6 mg/L). Ponds fertilized with RB received the greatest amount of organic fertilizer (Table 1) and had the lowest weekly dissolved oxygen as shown in Figure 5. Dissolved oxygen in ponds fertilized with DS was significantly higher on weeks 1, 4, and 5 and was higher in ponds fertilized with PD on weeks 1, 3, and 4 than that in RB ponds. Predicted low dissolved oxygen ($\leq 40 \%$ of saturation) required emergency aeration on 11, 8, and 4 nights for ponds fertilized with RB, DS, and PD, respectively.

There were no statistical differences in water temperature among





treatments. Water temperature averaged 20.7 °C; unseasonally cold mean water temperatures were experienced during week 2 (16.4 °C) and week 3 (17.9 °C).

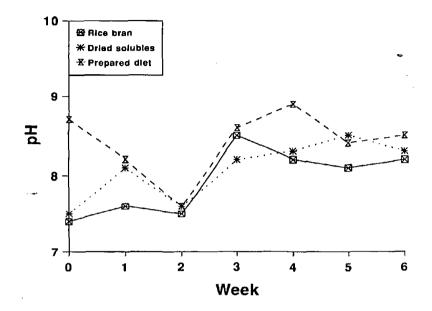
Mean ammonia and nitrite values were not significantly different among ponds fertilized with RB, DS, or PD over the culture period or by weeks. However, lower average levels of ammonia (0.12 mg/L) and nitrite (0.004 mg/L) over the culture period were observed for ponds fertilized with RB than those in ponds fertilized with DS or PD. Mean observed levels of ammonia and nitrite over the culture period were 0.31 and 0.16 mg/L for DS-fertilized ponds and 0.24 and 0.19 mg/L for PD-fertilized ponds, respectively.

DISCUSSION

Choice of organic fertilizers in ponds used to raise larval fishes should be based on local availability, cost, personal experience, and zooplankton response (Barkoh and Rabeni 1990). Mims et al.

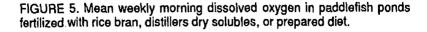
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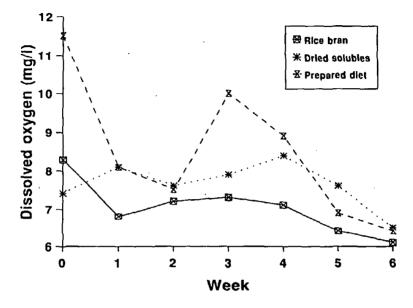
FIGURE 4. Mean weekly afternoon pH in paddlefish ponds fertilized with rice bran, distillers dried solubles, or prepared diet.



(1991) reported that RB met those criteria and should be used as the recommended organic fertilizer for production of juvenile (≥ 120 mm in TL) paddlefish. Rice bran also serves as a direct food source for cladocera (De Pauw et al. 1981), provides satisfactory and stable water quality, and reduces the incidence of filamentous algae (Mims et al. 1991). In the present study, higher survival and significantly greater yields were obtained in ponds fertilized with RB than those in ponds fertilized with DS. Differences in fish growth rates between the two by-products were found. Fish sampled during weeks 5 and 6 in ponds fertilized with RB had growth rates of 3.6 and 2.3 mm/day, respectively, compared to growth rates of 0 and 1.4 mm/day, respectively, in ponds fertilized with DS. Mims et al. (1991) reported that paddlefish growth rates ≥ 2.5 mm/day indicated availability of preferred food items.

Mean fish yield and survival in ponds fertilized with RB were lower than those previously reported by Mims et al. (1991). Lower





yield and survival in this study probably resulted from: colder water temperatures (20 vs. 24°C) and its effects on decomposition of organic matter by bacteria (Barkoh and Rabeni 1990); zooplankton generation time, longevity and reproduction (Allan 1976); and larval paddlefish growth (Mims and Schmittou 1989). The linear relationships of TL and weight over weeks suggest that maximum standing crops were not reached in any of the treatments. Semmens (1982) stocked 49,000 larvae/ha and fertilized with 600 kg/ha of alfalfa pellets and 300 kg/ha of meat/bone meal and harvested 125 kg of paddlefish/ha with an average TL of 100 mm in 40 days, which were lower and shorter than those obtained in this study.

Fish in ponds fertilized with PD had a greater mean weight per fish than fish in ponds receiving DS. The prepared diet could have served also as a supplemental feed. Paddlefish congregated around the feed and appeared to feed aggressively when PD was broadcast over the water surface. The same feeding response was not observed when RB or DS was applied. A combination of live organisms and prepared diets has resulted in greater larval growth and survival of common carp, *Cyprinus carpio* (Jhingrah and Pullin 1985), bighead carp, *Aristichthys nobilis* (Fermin and Rocometa 1988), striped bass, *Morone saxatilis* (Fitzmayer et al. 1986), and smallmouth bass, *Micropterus dolimieui* (Ehrlich et al. 1989), than have diets consisting either of live organisms or of prepared diets alone. A combination of live organisms and prepared diets has not been tested on larval paddlefish. Fitzmayer et al. (1986) have shown that supplemental feeding increased not only hybrid striped bass yield and survival, but that nutrients released from uneaten feed and fish feces by bacterial decomposition helped maintain zooplankton in the ponds for longer periods. Mims (1992) also reported higher *Daphnia* densities and biomass in PD ponds, compared to those in RB ponds in response to post-stocking fertilization.

The impact of fertilizers on water quality must also be considered when choosing an appropriate fertilizer. In general, water quality was better in ponds fertilized with RB than in ponds fertilized with DS or PD. Ponds fertilized with RB retained a brown coloration or stain during the 40-day period and had lower Secchi disk visibilities, reduced sunlight penetration, and filamentous algae growth compared to ponds fertilized with DS or PD. The reduction in filamentous algae is significant because filamentous algae are known to be detrimental to paddlefish survival (Mims et al. 1991). Alkalinity in ponds fertilized with RB was higher than in ponds fertilized with DS or PD. Higher alkalinity probably occurred because of the high organic load in RB ponds, with its increased bacterial decomposition, and produced greater amounts of carbon dioxide which, in turn, increased the solubility of calcium carbonate (Boyd 1990). All other water quality parameters were within acceptable ranges for paddlefish (Mims et al. 1991).

Rice bran cost \$63/MT, DS cost \$330/MT, and PD cost \$898/MT. Based on quantities applied in this study (Table 1), RB was the cheapest; it cost \$148/ha, compared to \$331/ha for DS and \$518/ha for PD. Despite higher paddlefish survival in ponds fertilized with PD, prices of juvenile fish, based only on the cost of fertilizer, were: \$0.004/fish for RB, \$0.011/fish for DS, and \$0.011 fish for PD. Therefore, juvenile paddlefish production in earthen ponds fertilized with RB was 2.8 times less expensive than production in ponds fertilized with PD or DS.

Experimental results support the findings of Mims et al. (1991) that RB is recommended over DS and PD as an organic fertilizer to stimulate *Daphnia* spp. production in paddlefish ponds, based on fish yields, impact on water quality, and cost. Data and observations indicate that PD may serve not only as a fertilizer to stimulate *Daphnia* spp. production but also as a supplemental feed. Further research should compare intensive larval paddlefish production in ponds, with prepared diets as a direct food source and in ponds with live organisms or a combination of both to increase fish production and survival.

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